Item 2. The scientific abstract

Information for Item 2. The scientific abstract is provided below.

An attenuated strain of *Salmonella typhimurium,* designated VNP20009, was generated by deletion of the *msbB* and *purI* genes. When VNP20009 was administered intravenously (IV) to mice bearing spontaneous, syngeneic, or human xenograft tumors, the bacteria accumulated preferentially within the extracellular components of tumors, forming tumor-to-normal tissue ratios exceeding 300-1000 to 1. VNP20009 was administered safely at doses up to $2.5x10^9$ cfu/kg in monkey toxicology studies. Based on the preclinical data, VNP20009 entered Phase I human clinical trials in November 1999, and has now been administered to >45 patients by IV or direct intra-tumoral injection. By the intra-tumoral route, a maximum tolerated dose has not been reached, and dose escalation continues past the current dose level of $4x10^7$ /m2. Furthermore, VNP20009 persisted in injected tumors for at least 2 weeks in 8/11 patients treated to date. By 30-min IV administration, a maximum tolerated dose (MTD) of $3x10^8$ cfu/m^2 has been established. In all patients treated to date, VNP20009 was not shed in urine or stool.

VNP20009 has been further modified by chromosomal insertion of an *E coli* cytosine deaminase (CD) gene at the *ΔmsbB* locus which, when expressed, converts 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). The CD containing VNP20009 was designated TAPET-CD or VNP20029. TAPET-CD had similar efficacy and safety in murine tumor models and similar safety profiles in animal toxicology studies, compared to its parent VNP20009. Specifically, TAPET-CD had a reduced virulence of >10,000 fold, when compared to the wild-type *Salmonella* strain. It was well-tolerated at doses up to $2x10^6$ cfu/mouse and $1x10^{10}$ cfu/monkey. After an IV or direct tumor injection to tumor-bearing mice, TAPET-CD reached tumor levels as high as 10^8-l0^9 cfu/gm. When compared to the accumulation in liver or spleen, the normal tissues with the greatest colonization of TAPET-CD, tumor-to-normal tissue ratios of TAPET-CD were 300-1000 to 1. TAPET-CD also caused tumor growth inhibition of >90% in several murine tumor models. When 5-FC was administered by intraperitoneal (IP) injection once or 3 times daily to tumor-bearing mice that had been pre-treated with TAPET-CD, high levels of 5-FU (reaching 20-40 μM/g) were detected in the tumor, with low or undetectable 5-FU levels in normal tissues (e.g., spleen, liver, etc.). Furthermore, co-administration of 5-FC and TAPET-CD in 4 different murine tumor models enhanced anti-tumor activity compared to the significant anti-tumor activity of TAPET-CD alone, further confirming the benefit of the inserted CD gene.

On the basis of the preclinical data, a Phase I clinical protocol is proposed in which advanced cancer patients will receive TAPET-CD by direct intra-tumoral injection and 5-FC. TAPET-CD will be administered on day 1. 5-FC will be given orally q8h daily beginning day 4 or when all toxicities of TAPET-CD have resolved to ≤grade 1, and continued for 14 days. Tumor tissues will be sampled to verify TAPET-CD colonization and to measure intra-tumoral 5-FC and 5-FU concentrations on day 8. A second sample of tumor tissue will be obtained between day 15-17 in selected patients to confirm the persistence of high levels of bacteria in tumor and to obtain a second measurement of 5-FC and 5-FU intra-tumoral concentrations. The TAPET-CD/5-FC treatment cycle will be repeated in appropriate patients on day 29.